THE CONTINUING ENIGMA OF CIGUATERA*

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ABSTRACT

Research on ciguatera fish poisoning has expanded significantly over the last decade. In large part, this increase in effort is due to the identification of several benthic dinoflagellates as the toxin producers, a discovery soon followed by a series of field and laboratory studies on their distribution, abundance, growth characteristics, and toxin production. Equally important have been advances in the analytical techniques and equipment needed to chemically characterize the toxins. Much of that work benefited significantly from the rapid progress in chemical research on the numerous other toxins produced by marine dinoflagellates.

Despite this surge in activity (summarized in the proceedings of four recent conferences or workshops: Ragelis, 1984; Salvat, 1985; Anderson *et al.,* 1985; this issue), the general state of knowledge on ciguatera remains relatively poor, both in terms of toxin chemistry and the physiological ecology of the causative dinoflagellates. Some important generalizations are gaining acceptance, but discrepancies and disagreements abound. One of the objectives of this review is to place the many recent papers on ciguatera in a current perspective that not only identifies common observations or conclusions, but also accentuates those areas that require more research effort to resolve disagreements or contradictions.

INTRODUCTION

In many tropical regions, it has long been known that consumption of certain coastal marine fishes can cause human illness and occasional death. The name "ciguatera" was given to this phenomenon by the Spanish, based on the belief that a marine turban snail (called "cigua" in the Caribbean) was responsible for poisoning settlers in Cuba. Reports of similar fish poisoning in the Pacific date back to the early 17th century (Banner, 1976). Today the term "ciguatera" refers to intoxications resulting from the ingestion of tropical and subtropical finfish, distinct from histaminic poisonings or those associated with the pufferfish (Halstead, 1967).

Morbidity statistics are highly unreliable due to the tendency of many individuals not to report such illnesses, the wide geographic distribution of many islands where the problem is endemic, and the variability in symptomology. Mean annual incidence of reported cases in the Pacific island region (excluding Hawaii and Australia) is about 1–4 cases per thousand population (Lewis, 1984; Yasumoto *et al.,* 1984). In the Caribbean, 4.2 cases per thousand were reported from St. Thomas in the Virgin Islands (Olsen *et al.,* 1984). These values can be scaled up by factors of 2–5 using estimates of the fraction of poisonings that are never reported. The resulting estimates and those from other affected areas indicate that ciguatera has been responsible for far more cases of human illness over the past eight years than any other kind of seafood toxicity associated with consumption of fresh marine organisms (Ragelis, 1984). As many as 10,000–50,000 individuals may be poisoned by ciguatera annually.

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* For reprints of the entire ciguatera workshop proceedings contact: New England Biolabs Foundation, P.O. Box 413, Wenham, MA 01984. Those interested in reprints of individual papers should contact the author directly.

Although rarely fatal, the intoxications can be extremely debilitating and in some cases can recur sporadically for years after the initial poisoning. However, the most important impact of ciguatera may well lie in its effects on small-scale fisheries for local consumption and for export. This is especially serious in those poor or densely populated islands where fish traditionally have been a primary source of protein. Reviews by Lewis (1984) and Olsen *et al.* (1984) examined the impacts of ciguatera on marine resource development in the Pacific and Caribbean regions, respectively. In both areas, ciguatera is considered one of the most important constraints to fisheries resource development, second only perhaps to the inadequate size of those resources relative to the additional demands expected with future population and economic growth.

The symptoms of ciguatera poisoning have been described in great detail in numerous publications (Bagnis, 1968; Bagnis *et al.*, 1979; Withers, 1982; Ragelis, 1984; Yasumoto *et al.*, 1984; Steidinger and Baden, 1985). Usually the illness begins with gastrointestinal inflammation, leading to severe dehydration and weakness and eventually cardiovascular and neurological distress. The most distinctive features of ciguatera are severe puritus, hot/cold reversal (the "dry ice sensation"), and tingling and numbness of the extremities. A distinctive feature of this illness is that the neurological symptoms can persist for months or even years, occasionally recurring in seemingly healthy individuals long after their recovery from the initial poisoning. It is also noteworthy that ciguatera symptoms are highly variable between individuals and between regions. These latter differences are due in part to the fishes consumed. For example, Bagnis (1968) associated gastrointestinal disorders with the consumption of herbivorous fish such as the surgeonfish, and cardiovascular and neurological symptoms with carnivores such as grouper or snapper. As will be discussed later, this polymorphism in clinical features indicates that several toxins are involved in ciguatera poisoning—some confined to the primary herbivore consumers and others being transferred through the food chain to the largest predators.

There are no established treatments for ciguatera patients, although injections of steroids, non respiratory depressants, antihistamines, antidiarrhetics, and vitamins seem to alleviate some of the symptoms (Yasumoto *et al.*, 1984). Native remedies involve treatments which rapidly purge the digestive tract (Lobel, 1979).

The Dinoflagellate Toxin Source

Despite the long history of ciguatera, the most probable source of the toxins, namely a group of benthic dinoflagellates, was only discovered within the last decade. Even now there is a degree of uncertainty as to whether the toxins isolated from ciguatoxic fish are the same as those produced by cultures of these dinoflagellates.

Prior to these recent developments, many theories implicated diseased fish, pollution, and other general phenomena in the poisonings. An exceedingly thorough examination of the feeding behavior of ciguatoxic fish in the Pacific by Randall (1958) led to a food chain theory whereby the toxin was presumed to be produced by a benthic microorganism (an unspecified alga, protozoan, fungus, or bacterium) which is first ingested by herbivorous fishes; the toxin is then transferred to larger carnivores. This theory proved to be remarkably accurate, although nearly 20 years passed before its validity was proven by the identification of the source organisms. The breakthrough occurred when Yasumoto *et al.* (1977b) found considerable toxicity in a sample of algae and detritus collected from the surface of dead coral in the Gambier Islands of French Polynesia. They also found high numbers of a large dinoflagellate in the most toxic samples and relatively few in low toxicity samples. The same pattern held for the stomach contents of high and low toxicity fish. Tentatively identified as

Diplopsalis sp., the organism was later placed in a new genus and named *Gambierdiscus toxicus* (Adachi and Fukuyo, 1979). To confirm the link between this dinoflagellate and fish toxicity, Yasumoto *et al.* (1977b) used various sieving and separation techniques to obtain dinoflagellate-rich fractions from heterogeneous detrital samples containing sand and coral fragments. Bioassays of the dinoflagellate samples showed that toxin content was directly proportional to the number of *G. toxicus* in the samples. Extracts from the dinoflagellate samples yielded two toxins, one with chemical and pharmacological properties identical, or closely related to ciguatoxin and the other resembling maitotoxin.

This study seemed to fix conclusively *G. toxicus* as the ciguatera elaborator, but the presence of many other co-occurring benthic dinoflagellates in the toxic samples and the detection of minor toxins of unknown origin in grazing herbivores and detritus feeders led Yasumoto *et al.* (1980) to test other dinoflagellates for toxicity. The results of their work and that of others (Nakajima *et al.*, 1981; Tindall *et al.*, 1984; Yasumoto, 1987) document the surprising fact that many of the dinoflagellates in tropical waters that live on or in close association with macroalgae or other surfaces are toxic. An example of the unexpected nature of these findings is that a survey of benthic dinoflagellates from Okinawa revealed toxins in all nine of the species examined (Nakajima *et al.*, 1981). Such results would never be expected in a similar survey of planktonic dinoflagellates, where toxicity is by far the exception rather than the norm. These results also add a degree of confusion to the ciguatera problem, since the existence of an array of toxins within an assemblage of organisms necessarily confounds the interpretation of chemical analyses and epidemiological surveys.

Three different types of toxins have been detected in the benthic dinoflagellates. *Gambierdiscus toxicus, Prorocentrum lima, P. concavum, Ostreopsis siamensis, O. ovata, Amphidinium carteri,* and *A. klebsii* all produce toxins which can kill mice (Nakajima *et al.*, 1981; Yasumoto, 1987). *Amphidinium carterii, A. klebsii, Coolia monotis,* and *P. rhathymum* (= *mexicanum*) produce toxins with strong hemolytic activity, but in fact some degree of hemolysis was observed using extracts of all nine species examined by Nakajima *et al.* (1981). *Prorocentrum concavum, A. carterii,* and *A. klebsii* produce strong ichthyotoxins; *P. concavum* is exceptionally potent. It should be stressed that not all of these toxins are involved in ciguatera. Although ichthyotoxins and hemolytic agents could have important effects on fish in tropical areas, only the species that produce toxins capable of killing mice will be considered further.

All of the work described above was based on cultures of dinoflagellates from the southern Pacific region. Subsequent investigations by Shimizu *et al.* (1982) confirmed the presence of *G. toxicus* in Hawaii. Similarly, surveys in the Caribbean by Tindall *et al.* (1984) indicated a species assemblage the same as that in the Pacific, including *G. toxicus* which produces ciguatoxin, one other lipid-soluble toxin, and maitotoxin. *Prorocentrum concavum* extracts were actually more potent than those from *G. toxicus* in that study.

The overall view that arises is that the benthic dinoflagellate community described above can be found throughout the world in tropical and subtropical regions where ciguatera is a problem. It is a diverse community consisting of species from at least four genera. All are photosynthetic, but they have little else in common other than their association with the benthos. Even within the benthos, they differ greatly in their habitat preference with some living attached to macroalgae and other surfaces, some in the sand, and the remainder free-swimming but still closely associated with surfaces. The reason that so many of these benthic dinoflagellates are toxic is a fascinating mystery that may be linked somehow to their habitat preference. Although this

is a clue that bears on the origins and functions of these toxins, elucidation of their role in dinoflagellate metabolism remains a distant but tantalizing goal.

TOXIN CHEMISTRY

Despite a concerted research effort over more than two decades, knowledge of the chemical characteristics and structure of the ciguatera toxins is incomplete. Reasons for this status are many: the toxins are present in extremely low concentrations in fish tissue; they can be unstable during the complex extraction and purification procedures; production of ciguatoxin in dinoflagellate cultures has been either minimal or non-existent; and reliable, sensitive assay methods specific for each toxin are not available.

The principal toxin in ciguatera poisoning is called ciguatoxin. This was first purified from red snapper in the Pacific (Scheuer *et al.*, 1967) and later from moray eel and shark flesh. Moray eel liver has been used extensively in subsequent studies because of its relatively high toxin content. The yield after extraction is still extremely low, however, as initial concentrations average only 10–20 ppb (Yasumoto *et al.*, 1984; Tachibana *et al.*, 1987). Ciguatoxin is insoluble in water or benzene, but readily partitions with methanol, acetone, ethanol, or 2-propanol.

The molecular structure of ciguatoxin has not yet been established, although ^{1}H NMR data suggest a molecular weight of 1111.7 ± 0.3 amu and a formula similar to $C_{53}H_{77}NO_{24}$ or $C_{54}H_{78}O_{24}$ (Tachibana *et al.*, 1987). The most probable configuration is that of a highly oxygenated long-chain fatty acid in which most of the oxygen atoms occur as cyclic ether linkages. This latter observation is consistent with the similar behavior of ciguatoxin and okadaic acid in thin layer chromatography (Murakami *et al.*, 1982) and with the cross-reaction of ciguatoxin and other polyether toxins in immunoassays (Baden *et al.*, in prep.; Hokama *et al.*, 1987).

Two additional toxins can be extracted from ciguatoxic fish, one of which is ether-soluble like ciguatoxin and the other water-soluble. The former has been called scaritoxin (Bagnis *et al.*, 1974) because it is found predominantly in many species of parrotfish (*Scarus*). It is easily separated from ciguatoxin on a DEAE-cellulose column and migrates differently in thin layer chromatography (Chungue *et al.*, 1977). No scaritoxin could be detected in the diet of the parrotfish (Yasumoto *et al.*, 1977a), yet flesh samples clearly contained the toxin. The presence of ciguatoxin as the dominant toxin in the gut and liver of the parrotfish was a further indication that scaritoxin is not a naturally occurring toxin in the fish's diet but instead is a metabolite of ciguatoxin (Yasumoto *et al.*, 1977a). This hypothesis was recently confirmed by the demonstration that ciguatoxin and scaritoxin can be reversibly interconverted by manipulation on basic alumina columns (Tachibana *et al.*, 1987).

Looking back to the assumed polyether structure of ciguatoxin with its many hydroxyl groups, it now seems reasonable that hydrogen-bonding at various locations could yield compounds with distinct chromatographic and pharmacological characteristics but the same general structure as the parent ciguatoxin (Tachibana *et al.*, 1987). Such changes could readily occur within fish following consumption of the dinoflagellate.

The second major toxin involved in ciguatera poisonings is maitotoxin, originally isolated from the surgeonfish *Ctenochaetus striatus* (Tahitian name, "maito") and subsequently found in significant quantities in extracts of cultures of *G. toxicus* and possibly *P. concavum* (Tindall *et al.*, 1984). Maitotoxin is more polar than ciguatoxin and is thus soluble in water. Its occurrence is thus limited to the viscera of herbivores or benthic grazers in contrast to the lipid-soluble ciguatoxin which can accumulate

in flesh and move through the food chain. Although maitotoxin is produced in abundance in dinoflagellate cultures, it remains poorly characterized. Purified material yields an amorphorus white solid whose molecular weight is thought to be around 3300 amu (Yasumoto, 1987). There are no amino acid or fatty acid moities in the molecule, and there appear to be no chemical similarities between maitotoxin and ciguatoxin (Yasumoto *et al.*, 1984).

A third toxin which may be involved in ciguatera poisonings is okadaic acid, a polyether fatty acid derivative first found in sponges. This lipid-soluble compound has been isolated from *P. lima* (Murakami *et al.*, 1982), a dinoflagellate included in the benthic ciguatera community. Okadaic acid and structurally similar compounds have been implicated in diarrhetic shellfish poisoning (DSP), most commonly due to planktonic dinoflagellates of the genus *Dinophysis* (Yasumoto, 1985). Symptoms following the consumption of shellfish containing these compounds include diarrhea, vomiting, and other gastrointestinal disorders. Since similar symptoms have been reported for some ciguatera poisonings and since *P. lima* is present in the seaweeds grazed by herbivorous tropical fish, it is possible that okadaic acid is causing one type of illness among several grouped under the general term "ciguatera."

One intriguing aspect of recent work on toxin chemistry is that ciguatoxin production has been extremely low in laboratory cultures of *G. toxicus*, even when strains isolated from highly toxic wild material are used (Yasumoto *et al.*, 1979b; Bagnis *et al.*, 1980). A lack of detectable ciguatoxin in wild *G. toxicus* populations also has been observed (Gillespie *et al.*, 1985). There are numerous reports of lipid-soluble material from culture extracts that kill mice (*e.g.*, Yasumoto *et al.*, 1977, 1979b; Withers, 1982; Tindall *et al.*, 1984; Durand-Clement, 1987), but the lack of assay methods that distinguish ciguatoxin from maitotoxin leaves a cloud of uncertainty over such results. Some workers believe that traces of maitotoxin can remain in the lipid soluble "ciguatoxin" fraction and thus result in mouse mortality even when ciguatoxin is absent (Gillespie, pers. comm.). Ciguatoxin is readily separated from maitotoxin through the use of a silicic acid column and a stepwise elution with chloroform and methanol (Tachibana, 1980). Ciguatoxin elutes with chloroform: methanol at 9:1 and maitotoxin at 1:1. This procedure has not been used routinely by all workers, however, so the problem of residual maitotoxin remains a potentially important artifact in many studies. A more complicated and cautious approach to studies of this kind is that of Baden *et al.* (1985), who supported their claim of production of a ciguatoxin-like compound in *G. toxicus* cultures by demonstrating that their lipid-soluble extract contained a sodium channel depolarizing toxin whose effect on a crayfish giant axon could be partially blocked by tetrototoxin. This type of assay, or the column separation scheme described above, would seem to be necessary prerequisites for all work directed at the characterization or measurement of ciguatoxin; yet such has not generally been the case.

The state of the chemical characterization of the ciguatera toxins can be summarized as follows. It is clear that several toxins may be responsible for the poisonings. Ciguatoxin, the primary toxin, has been isolated from larger carnivores, but is only partially characterized because of an inadequate supply of purified material. Although considerable circumstantial evidence has been compiled linking *G. toxicus* to this toxin, it has not yet been conclusively demonstrated that the toxin produced by the dinoflagellate is either identical to, or is a direct precursor to the ciguatoxin accumulating in the fish. Scaritoxin, another lipid-soluble toxin detectable in fish flesh is presumably a metabolite of ciguatoxin, apparently formed after the fish has ingested the primary toxin. Maitotoxin is the most readily available toxin since it is produced in abundance in *G. toxicus* cultures, yet its chemical structure also remains

unknown. The extremely high potency of maitotoxin, and the likelihood that trace quantities of it remain in the lipid fraction of many separation schemes, makes it difficult to interpret earlier studies, especially those claiming ciguatoxin production in *G. toxicus* cultures. References to extracted compounds as "ciguatoxin-like" or "maitotoxin-like" abound in the current literature, underscoring the analytical uncertainties that remain in this field despite years of concerted research effort. Okadaic acid, the final toxin of concern here, has been well-characterized chemically but has not been shown to be directly involved in fish poisonings. Its inclusion in this discussion is based on the similarities between symptoms associated with this toxin as a cause of diarrhetic shellfish poisonings and those from certain ciguatera poisonings, as well as on the proven production of this compound by *P. lima,* a prominent species within the ciguatera dinoflagellate community.

PHARMACOLOGY

The suite of symptoms associated with ciguatera poisonings is due in part to the wide variety of fishes consumed and the diversity of toxins within those fishes. In addition, pharmacological studies on extracted toxins are subject to the same artifacts discussed earlier due to variability in sample purity. Nevertheless, a coherent picture of the effects of the ciguatera toxins on living systems is beginning to emerge.

Both ciguatoxin and maitotoxin are among the most potent marine toxins known, having LD_{50}'s of 0.45 and 0.13 $\mu g \ kg^{-1}$ [intraperitoneal (i.p.), mouse] respectively (Tachibana, 1980; Yasumoto, 1985). Bagnis *et al.* (1987) used bioassays of leftover portions of fish that had caused ciguatera poisonings to derive a relationship between oral dose and ciguatera symptoms in humans. The extreme potency of ciguatoxin determined from intraperitoneal injections in mice was still evident in terms of human oral potency, with a mean dose for 50% illness at 2 ng kg^{-1} and a minimum lethal dose estimated to be 20 ng kg^{-1}. The primary action of ciguatoxin now appears to be a depolarization of the sodium channel, an effect that can be blocked by application of tetrototoxin (Rayner 1970; Rayner and Kosaki, 1970; LeGrand and Bagnis, 1984). Scaritoxin also has been shown to have a depolarizing action on excitable membranes and generally seems to have a pharmacological mode of action close to that of ciguatoxin. In hindsight, this is to be expected since it is now clear that the two compounds are structurally related. Li (1965) reported that ciguatoxin isolated from several fish species functioned as an anticholinesterase, but this contention was tested by Rayner *et al.* (1969) who concluded that there may be some inhibition of cholinesterase in *in vitro* preparations but that this was not an effect of ciguatoxin in living organisms.

Maitotoxin also acts as a neurotoxin, but its effects are most probably centered on the calcium channel. Neurophysiological studies (Takahashi *et al.,* 1982, 1983; Ohizumi *et al.,* 1985; Miller and Tindall, 1985; Ohizumi, 1987) indicate that maitotoxin causes positive inotropic effect on smooth muscle, suggesting that the toxin causes an increase in Ca^{2+} permeability, probably through calcium channels. This action is not affected by treatment with tetrototoxin or by excess sodium.

The same functions that make the ciguatera toxins potent marine poisons also makes them potential tools in the study of excitable membranes. The utility of saxitoxin and tetrototoxin as molecular probes is already well established (Caterall, 1985), but the active use of ciguatoxin and maitotoxin in similar neurophysiological studies only awaits the increased availability of purified material.

ASSAY METHODS

Ciguatera toxins are odorless, tasteless, and generally undetectable by any simple chemical test, so bioassays traditionally have been used to monitor suspect fish. Many

native tests for toxicity in fish have been examined, including discolorations of silver coins or copper wire or the repulsion of flies and ants, but all of these were rejected as invalid (Banner, 1964).

Oral feeding of fish to cats is a simple and sensitive assay, but has the disadvantage that the cats often regurgitate part of the meal. Since the mongoose does not regurgitate and thus exhibits a response that is related to the amount of toxin ingested, a roughly quantitative assay was designed and used extensively in Hawaii using trapped wild animals (Banner, 1976).

Feeding tests such as those above are useful in screening fish for toxicity, but they are non-quantitative and cumbersome. As is common with other dinoflagellate toxins, a mouse bioassay was developed, but this procedure required purification of fish extracts since mice are relatively insensitive to ciguatoxin (Yasumoto et al., 1971). The mouse bioassay has been used in numerous surveys in the Pacific and is described in detail in Yasumoto et al. (1984).

One alternative to the use of mice is the mosquito bioassay which was recently used by Bagnis et al. (1987) to obtain a dose-response relationship between ingested ciguatoxin and clinical symptoms in man. The mosquito assay correlates reasonably well with cat and mouse bioassays, and has the additional advantages that it is rapid, dependent on a simple extraction, and requires only a small amount of fish for analysis.

All bioassay methods have common disadvantages, perhaps the most important of which is the lack of specificity for individual toxins. Several alternative methods are now under development that have the potential to provide the needed sensitivity and specificity. One is a radioimmunoassay for ciguatoxin originally developed in Hawaii (Hokama et al., 1977). During a two-year study, this method was used to screen amberjacks (*Seriola dumerili*) on the Hawaiian market, 15% of which were rejected (Kimura et al., 1982). No poisonings were reported from that fish species during the study, although other untested species did cause illness. Despite this success, the radioimmunoassay is too costly and time-consuming for routine use and does cross-react with okadaic acid and other polyether compounds. An inexpensive, rapid colorimetric enzyme immunoassay was then developed (Hokama et al., 1983) which was subsequently adapted further to what is now called the "stick test" (Hokama et al., 1987). This technique, which uses small, coated bamboo sticks to assay the fish flesh, shows great promise since each assay takes less than 15 minutes and the procedures are sufficiently simple to be employed in the field. One disadvantage, however, is that the antibody reacts with okadaic acid, brevetoxin, and other polyether compounds with structures similar to ciguatoxin. It is hoped that ongoing attempts to develop monoclonal antibodies to each of these closely related polyethers will allow the "stick test" to attain the necessary degree of specificity. The importance of this assay should not be discounted even in its present form, however, since the cross-reaction problems seem to generate false positives (*i.e.,* rejection of fish that are safe to eat) but very few false negatives. This clearly seems to be the direction of choice for future work on assay development.

FIELD ECOLOGY

Dinoflagellate/host specificity

The ciguatera dinoflagellates are all considered benthic, epiphytic, or metaphytic—living attached to or in close association with sand, coral, macroalgae, and other surfaces. Table I lists the macroalgal species found associated with high concentrations of *G. toxicus*. Most of these host algae are branched or tufted in form as sug-

Macroalga genera with epiphytic Gambierdiscus toxicus[1]

Green algae	Red algae
CHLOROPHYTA	RHODOPHYTA
Caulerpa	*Acanthophora*
Chaetomorpha	*Amphiroa*
Cladophora	*Asparagopsis*
Codium	*Digenia*
	Galaxura
Brown algae	*Gelidium*
PHAEOPHYTA	*Hypnea*
Dictyota	*Jania*
Sargassum	*Laurencia*
Turbinaria	*Pterocladia*
	Spyridea

[1] Data compiled from the Caribbean and tropical Pacific from: Yasumoto *et al.,* 1977; Shimizu *et al.,* 1982; Whithers 1982; Taylor and Gustavson, in press; Carlson *et al.,* 1984; Carlson and Tindall, 1985; Carlson 1984; Taylor 1985; Bagnis *et al.,* 1985; Gillespie *et al.,* 1985.

gested by Taylor (1985), but *G. toxicus* will also attach to most kinds of algae regardless of structure (Gillespie *et al.,* 1985) while avoiding bare coral substrate and seagrass blades (Carlson and Tindall, 1984).

Algae which persist on coral reefs in the presence of herbivores usually are structurally tough or distasteful (*e.g., Halimeda, Penicillus, Caulerpa,* etc.). Delicate filamentous algae which are readily eaten by many herbivorous fishes are rare and usually appear on new bare patches of rock and coral (*e.g., Polysiphonia, Enteromorpha,* etc.). Because filamentous algae are rare, they have been thus far undersampled for *G. toxicus* occurrence. Randall's (1958) early insight into ciguatera ecology considered whether outbreaks occur when reef surfaces were bare. The question remains whether under these circumstances the first colonizing filamentous algae might also be epiphytized by *G. toxicus.* Many of the host algae in Table I persist as macrophytes either by living in habitats or zones where herbivory is low or by producing secondary metabolites which inhibit fish feeding (Hay 1984, 1985; Hay and Goertemiller, 1983).

Therefore, the occurrence of *G. toxicus* on certain of these macroalgae may not be a good indicator of their importance in the transfer of toxins to higher trophic levels. The transfer actually may occur through grazing on the less abundant, undersampled macroalgae which are preferred foods. In other words, the persistence of macroalgae with epiphytic *G. toxicus* may only be an indication of what is *not* being eaten, with the real uptake of the toxin occurring as less abundant, smaller algae are cropped by the herbivores. This is analogous to the nutrition of phytoplankton in the central oceans where essential nutrients like nitrogen and phosphorus are below analytical detection limits but are, nevertheless, available through rapid recycling or small-scale patchiness. Clearly, despite the numerous studies which have enumerated the host macroalgae for the epiphytic dinoflagellates, an understanding of the reasons for these associations is far from complete. Suggestions of host selectivity based on form and structure may be valid (Taylor, 1985; Taylor and Gustavson, in press) but must remain speculation until controlled experiments are conducted.

Fish herbivory

Herbivorous fishes comprise a diverse taxonomic assemblage of species and possess widely different capabilities for utilizing plants as food (Lobel, 1981). An impor-

tant uncertainty is the relationship between ciguatera toxicity and fish digestive mechanisms and feeding selectivity.

Certain herbivorous fishes are well-known for morphological specializations enabling trituration, such as parrotfishes (Scaridae) with a bony pharyngeal mill and certain surgeonfishes (Acanthuridae, *e.g., Ctenochaetus* spp.) with a muscularized, gizzard-like stomach. Another mechanism used by some marine fishes for rupturing ingested plant cells—lysis by gastric acidity (pH range 2.4–4.3)—recently has been described (Lobel 1981). Fishes with acidic stomachs include certain surgeonfishes of the genus *Acanthurus* and the territorial herbivorous damselfishes [Pomacentridae; *Stegastes* (= *Eupomacentrus*) spp.]. Utilization of plant foods by fishes is apparently limited to these three digestive mechanisms. Fishes are not known to produce cellulase or other enzymes capable of digesting plant cell walls. However, they do produce several carbohydrases capable of digesting plant cell contents (Kapoor *et al.,* 1975). An intestinal microorganism has been found recently in the gut of two herbivorous fishes in the Red Sea but was absent from the guts of several other species of the same family (Acanthuridae; Fishelson *et al.,* 1985). This microorganism probably does not have a primary role in digestion (Fishelson *et al.,* 1985) and none have been identified in other herbivorous fishes (Kapoor *et al.,* 1975).

Herbivorous fishes are classed as "browsers" or "grazers" (Jones, 1968). Grazers ingest substantial quantities of sand and coral particles while feeding on algae by either rasping the substrate or sucking loose grains. Browsers bite or tear algae and rarely ingest any inorganic material. Herbivorous marine fishes are further characterized by three general types of alimentary morphology: (1) an elastic stomach capable of secreting strong acids (pH 2.4–4.3), with a long intestine, (2) a thick-walled, gizzard-like stomach (pH 6.3–7.9) and a medium length intestine and, (3) a bony pharyngeal mill with no stomach present (anterior intestine pH ∼ 8.4) and a relatively short intestine (Lobel, 1981). The gizzard-like stomach and the pharyngeal mill are characteristic of grazers. Fishes with an acidic stomach are browsers. For details see Lobel (1980, 1981) and Lobel and Ogden (1981).

It is unknown how these different digestive capabilities may relate to ciguatera toxicity, but it has been shown that the surgeonfish, *Ctenochaetus striatus* (type 2) and parrotfish species (type 3) have distinct toxin characteristics as described previously (Bagnis *et al.,* 1974; Yasumoto *et al.,* 1984). These fishes are also the most frequently implicated in ciguatera poisoning while fishes belonging to type 1, such as the Pacific surgeonfish, *Acanthurus triostegus,* are of lower risk. The relationships between this pattern and the fishes' feeding habits or the possible interaction of ingested dinoflagellate and fish gut chemistries remain obscure.

Few studies have quantified the preference by fishes for particular algal species (reviewed by Ogden and Lobel, 1978). It has been more common to assess survivorship of transplanted algae exposed to the ensemble of reef herbivores (*e.g.,* Earle, 1972; Hay, 1984; 1985). Analysis of stomach contents in herbivorous fishes is difficult because some species completely grind their food. Gut contents show only what has been eaten, do not necessarily reflect preferences, and can be further confounded by the relative indigestability of some algae over others. Nevertheless, many studies show that certain algae are much more likely to be eaten than others, including several known hosts to *G. toxicus* (Table I; Earle, 1972; Ogden and Lobel, 1978; Hay, 1984; 1985).

Browsers consume fine filamentous algae and epiphytes. Fishes of this type include the surgeonfish *Acanthurus triostegus* (Acanthuridae, Randall 1961) and the territorial damselfishes, *Stegastes* spp. (Pomacentridae, Lobel 1980). These damselfishes feed specifically on epiphytes overgrowing small red algal thalli (Lobel, 1980).

Predator consumption of herbivorous fishes in the Caribbean[1]

Herbivore	% predator spp. having eaten this prey (n = 58 spp)	% of fish individuals eaten by all predators (n = 391 ind.)
Grazers		
Scaridae	28%	13.3%
Monocanthidae	21%	3.8%
Acanthuridae	14%	2.3%
Browsers		
Pomacentridae	10%	3.4%

[1] Data from Randall, 1967.

These fishes have not yet been assayed for ciguatera toxicity probably because damsel-fishes are small and not eaten by people. It seems, however, that they are prime candidates because *G. toxicus* is frequently epiphytic on red algae, and they are important in the trophic linkage to higher carnivores (Table II).

Grazers with a gizzard-like stomach consume microalgae mixed with fine sand and detritus. This group includes the herbivorous fish, *Ctenochaetus* spp., frequently implicated in ciguatera fish poisoning (Randall, 1980). Some species of this group have solid cropping teeth and are able to bite a variety of small algal thalli, but *Cteno-chaetus* is distinct. This fish has numerous, very elongate teeth with expanded in-curved tips which are loosely attached in the jaw (Randall, 1955). *Ctenochaetus* spp. feed on fine particulate material. *C. strigosus* in Hawaii contained up to 90% fine inorganic sediment with the rest being unicellular algae, small fragments of filamen-tous algae, and detritus (Randall, 1955; Jones, 1968). Randall (1955) related the fol-lowing account of *C. strigosus* feeding: "When a thallus of fine filamentous red algae (*Polysiphonia* sp.) was placed in an aquarium . . . the fish attempted to feed upon it. Their slender movable teeth, not able to effectively bite off pieces, soon became entangled in the alga, resulting in very little being ingested." However, the fish was able to feed on fine particles of the alga that settled on the bottom. It sucked up particulate algae with very fine sediment. Large sand grains were generally avoided. According to Carlson and Tindall (1985), *G. toxicus* is rarely found on sand, thus it would seem that if it is eaten by *Ctenochaetus,* it must be sucked off the surfaces of macroalgae.

The herbivorous fishes having a bony pharyngeal mill are parrotfishes (Scaridae). Adult reef parrotfish graze algae overgrowing dead coral surfaces and ingest quantities of calcium carbonate with their algal food (Randall, 1967, 1974; Ogden, 1977). Juve-nile parrotfish scrape fine filamentous algae and epiphytes from a variety of surfaces. When possible, they will feed on most kinds of the algae (*e.g.,* Earle, 1972) on which *G. toxicus* is epiphytic (Table II). Thus, despite the toughness or "bad taste" of certain host macroalgae, *G. toxicus* could be removed from these surfaces by parrotfishes. Controlled feeding preference experiments have demonstrated that one species, *Spar-isoma radians,* will eat seagrass blades with epiphytes in preference to bare blades (Lobel and Ogden, 1981). Parrotfish are the dominant family by weight on many tropical reefs and are the most common herbivore prey of large piscivores (Table I. Randall, 1967, 1974).

Once again generalizations concerning ciguatoxic fishes and their food habits are not yet possible given available field data. It is clear that numerous herbivores can be

toxic and that the toxin can easily move to higher food chain levels through predation. What is unclear is how the toxin is obtained by herbivores with such varied feeding habits and preferences. Perhaps the colonization of many different algal surfaces by *G. toxicus* ensures that it will enter the food chain through herbivorous fishes. But whether this happens as a continuous process, or sporadically when *G. toxicus* occurs on certain algae which rapidly colonize new reef surfaces and are then eaten by fishes, remains moot.

General habitat

As discussed earlier, the ciguatera dinoflagellates are found in tropical waters throughout the world, but there is a general pattern to this distribution. In both the Pacific and the Caribbean, for example, ciguatera seems to be restricted to islands and is not found along continental margins. It is also apparently lacking in the waters of the islands of the Western Pacific (Banner, 1976). Exceptions to this generality include Florida and the Great Barrier Reef of Australia. However, the region of Florida that is affected is along the Keys and eastern coast which are subject to intrusions of oceanic water; in Australia, toxic fish are found predominantly around the offshore reefs and not along the continental margin (Banner, 1976). This general "oceanic" scenario was confirmed in a survey of 86 locations on 15 Caribbean islands by Taylor and Gustavson (in press), who generalized that *G. toxicus* is absent from nearshore localities on large, high islands or major land masses with substantial land runoff, but thrives in areas most exposed to oceanic waters, notably near offshore outcrops or on the windward side of islands.

Within a region where the ciguatera community occurs, certain generalizations are emerging as to habitat preference, but interestingly, these generalizations sometimes differ between the Pacific and the Caribbean. For example, based on numerous surveys of islands in the Pacific, Yasumoto and co-workers (1979a, 1980) indicate that *G. toxicus* was most abundant in relatively high energy environments—exposed reef areas and turbulent channels. In contrast, an extensive survey in the Caribbean by Carlson (1985) showed much greater abundance of this species in protected lagoons and other inshore stations compared to reef stations. This observation seems to conflict with other reports that reef fishes in that region are very toxic, but the close proximity of reefs and lagoons in the Virgin Islands allows fishes to move freely between the two locations for feeding.

There are several possible reasons for the disparity in habitat preferences described above. Inadequate sampling might be one explanation, since the epiphytic dinoflagellates are notoriously patchy even on spatial scales of a few meters (Yasumoto *et al.,* 1979a; Taylor and Gustavson, in press). Another factor might be related to the season of the sampling, since it is now a relatively common observation (discussed below) that dinoflagellate abundance can vary significantly over the year at certain stations, especially those exposed to storm and wave activity. Surveys conducted over a short interval at one time of the year might not be representative of the species distribution at other times. Whatever the reason for this discrepancy in habitat preference, it is clear that accurate descriptions of the field distribution of the ciguatera dinoflagellates are difficult to obtain, but are nevertheless extremely important.

There is general agreement on other aspects of the field distributions of *G. toxicus*. Workers in both the Pacific and the Caribbean have observed that *G. toxicus* does not occur at shallow depths or in areas with high light intensities (Yasumoto, 1978; Yasumoto *et al.,* 1980; Carlson, 1985; Taylor and Gustavson, in press). Carlson (1984) found that macroalgal-associated dinoflagellates were generally not found at

depths less than 0.5 m where light levels exceeded 6.5×10^4 lux. Furthermore, dino-flagellate abundance was low in areas with white, sandy bottoms where light reflected from the bottom nearly equaled the incident irradiance. There is also general agreement that *G. toxicus* prefers high salinity water, being very scarce near the mouths of rivers or in areas of high runoff (Yasumoto *et al.,* 1980; Carlson, 1985; Taylor, 1985).

Long and short-term fluctuations have been observed in both the incidence of fish poisonings (Halstead, 1967; Banner, 1976) and the abundance of the ciguatera dinoflagellates. In Australia, *G. toxicus* cell numbers were shown to increase dramatically in September and October during two years of observations (Gillespie *et al.,* 1985). In the Virgin Islands, a similar periodicity in cell number was observed, with two peaks in abundance during twelve months of data (Carlson and Tindall, 1985). Although their data were more qualitative, Taylor and Gustavson (in press) noted seasonal fluctuations in *G. toxicus* abundance in Barbados. Relatively few environmental parameters were monitored during these studies, so it is difficult to speculate on the cause of the cyclical abundance. In all cases, however, low dinoflagellate abundance occurred during the periods when storm and wave activity were maximal. Stresses from wind and waves on the macroalgae are clearly reflected in the abundance of the dinoflagellate epiphytes. Carlson and Tindall (1985) also found a strong positive correlation between fluctuations in the numbers of toxic benthic dinoflagellates (including *G. toxicus*) and Virgin Islands' rainfall.

Several workers have looked for correlations between the fluctuating abundance of the ciguatera dinoflagellates and major nutrient concentrations, but without success. Yasumoto *et al.* (1980) found no relationship between inorganic phosphorus, total phosphorus, nitrite, nitrate, silicate, iron, dissolved organic carbon, and vitamin B_{12} and *G. toxicus* cell concentrations in French Polynesia. These water samples were taken in the general vicinity of the macroalgae used for the *G. toxicus* counts. Carlson (1984) did nutrient analyses on water collected immediately adjacent to the macroalgae. Both phosphates and nitrates were significantly correlated with three predominant dinoflagellates (*G. toxicus, P. concavum,* and *P. lima*), but no single limiting nutrient was identified. These results are consistent with the view that these epiphytic dinoflagellates may specifically associate with macroalgae where high concentrations of nutrients are available for growth (Steidinger, 1983).

One popular notion about ciguatera is that it can arise in previously unaffected areas or become worse in areas with a long history of low-level toxicity—all in response to disruption or destruction of reef surfaces (reviewed by Randall, 1958; Banner, 1976). The concept is that freshly denuded surfaces on a reef are colonized by certain opportunistic species of macroalgae that are ideal hosts for the epiphytic dinoflagellates. Thus dredging, shipwrecks, hurricanes, and other man-made or natural disturbances can all create the new surfaces needed for colonization. Support for this theory comes from Cooper (1964) who related toxicity in the Gilbert Islands to the locations of wrecks and anchorages, by Bagnis (1969) who reported an outbreak of poisonings at the previously non-toxic atoll of Hao after major changes to the reef system, and by Bagnis *et al.* (1985) who document a decrease in toxicity in the Gambier Islands in the years following an initial flare-up which followed soon after major reef destruction. There are many other reports that support this hypothesized link between "new surfaces" and toxicity, but there are also many instances where such events were not associated with increases in toxicity. Banner (1974) points out that the blasting of channels in the Gilbert Islands, typhoon flooding in Fiji, dredging at Johnston Atoll, and even reef devastation by the starfish *Acanthaster* were not followed by toxicity. Free or "new" coral surfaces may well provide an excellent mechanism for the accumulation of ciguatera dinoflagellates, but there are clearly other factors that must be suitable as well if an outbreak is to occur.

Culture Studies

Several ciguatera dinoflagellates have been studied in laboratory cultures, but the results obtained by different workers are sometimes confusing and contradictory. There is general agreement, however, on the temperature tolerance of *G. toxicus,* the only species for which data are available. Pacific strains of *G. toxicus* grow optimally near 27°C (Yasumoto *et al.,* 1984; Durand-Clement, 1987) but stop growing or die at temperatures above or below 30 and 20°C, respectively. The light tolerance of *G. toxicus* was examined by Yasumoto (1978) and Durand-Clement (1987) and interestingly, the relatively low light intensities reported for optimal growth (about 4000 lux) are very similar to field measurements of the light environment in Caribbean lagoons where Carlson (1984) found the highest numbers of this species.

Durand-Clement (1987) reports that *G. toxicus* growth was very poor in continuous light, but Carlson *et al.* (1984) successfully used continuous light for all of their culture studies on the ciguatera community. Similar discrepancies arise when growth data from bacteria-free cultures are compared. Durand-Clement (1987) found enhanced growth of *G. toxicus* (and a substantial decrease in the normally copious mucilage production) when bacteria were eliminated. However, Yasumoto *et al.* (1984) and Hurtel *et al.* (1979) both report that growth of this same species was markedly retarded in axenic culture, a finding similar to that of Carlson (1984) for *P. concavum.*

The benefits accruing to the ciguatera dinoflagellates from their close association with macroalgae remain entirely speculative and include enhanced nutrient availability, shading from dangerously high light intensities, and protection from turbulence. The first of these issues was examined by Carlson *et al.* (1984) in a detailed series of experiments testing the effects of various macroalgal and soil extracts on the growth of three of the ciguatera dinoflagellates. Growth of *G. toxicus* (in bacterized cultures) was enhanced by both soil extract and aqueous extracts of the macroalga *Chaetomorpha. Prorocentrum concavum* growth was stimulated by these same additions and by extracts of two other macroalgae, whereas *P. rhathymum* was inhibited by all such additions. Yasumoto *et al.* (1984) and Durand-Clement (1987) also report enhanced growth of *G. toxicus* following additions of soil extract, but other workers have reported inhibition of this species (Hurtel *et al.,* 1979).

One striking aspect of these results is that they are reminiscent of the state-of-knowledge about phytoplankton culture media prevailing 75 years ago (Pringsheim, 1912). In those days, phytoplankton growth in laboratory cultures was shown to require the addition of soil extract, ground-up copepods, or other poorly defined organics to the seawater base. It was subsequently shown by Provasoli *et al.* (1957) that the soil extract could be replaced by synthetic chelators (EDTA, NTA) and trace metals (iron, copper, zinc, manganese, cobalt, molybdenum). Progress in recent years has been even more dramatic, with chemically defined culture media being used to characterize the trace metal sensitivities (both toxic and nutritional) of a variety of phytoplankton species (reviewed by Huntsman and Sunda, 1980).

An hypothesis that follows from the above observations is that *G. toxicus* thrives in seawater that is oceanic rather than neritic in its chemical composition. One way to test this hypothesis would be to quantify the ciguatera dinoflagellates' sensitivities to, and requirements for, trace metals such as copper, zinc, manganese, and iron as has been done for other phytoplankton (Sunda and Guillard, 1976; Anderson and Morel, 1978; Brand *et al.,* 1983). Recent data indicate order of magnitude differences in zinc, manganese, and iron concentrations between oceanic and coastal waters (Bruland and Franks, 1983; Gordon *et al.,* 1982). Furthermore, Brand *et al.* (1983) showed that neritic species had significantly higher requirements for zinc and iron than oceanic species, which, when compared with measured concentrations of these

trace elements in natural waters, suggested that the metals may be as important as nitrogen, phosphorus, and silicon in regulating marine ecosystems. In this context, note the recent demonstration by Entsch *et al.* (1983) that iron is a limiting nutrient for primary producers in Australian coral reefs.

The different responses of the ciguatera dinoflagellates to macroalgal and soil extract additions may be related to the spatial scale of their association with macroalgae. Both *G. toxicus* and *P. concavum* live attached to algal surfaces, suggesting a need for the organic substances and other nutrients commonly exuded by macroalgae (Steidinger, 1983). The growth of those two species was clearly stimulated by soil and macroalgal extracts. In contrast, *P. rhathymum* is most commonly reported to be free-swimming and thus may be adapted to water with different chemical characteristics than that immediately adjacent to macroalgae. *Prorocentrum rhathymum* growth was inhibited by macroalgal extracts and grew well in artificial seawater medium that could not support *G. toxicus* and *P. concavum* growth without additions of soil extract (Carlson *et al.,* 1984).

These reports of growth stimulation of *G. toxicus* by soil extract are unfortunately inconsistent with the general perception that this species does not thrive in areas subject to terrestrial runoff (Taylor, 1985). The beneficial effects of the poorly defined macroalgal extract additions to laboratory cultures described above may be indicative of a specialized nutritional interaction between the dinoflagellates and their host algae, but laboratory-prepared soil extract should be functionally similar to the material carried to coastal waters by terrestrial runoff. As suggested by Taylor (1986), it may be that the extraction and sterilization procedure used to obtain soil extract in the laboratory somehow alters its chemical characteristics and inactivates potentially toxic components. Whatever the reason, much work will be needed to define the trace metal and organic requirements of the ciguatera community if the apparent conflicts between field and laboratory observations are to be resolved. This is an appropriate time to apply established trace metal methodologies to the ciguatera dinoflagellates, since only through controlled manipulations of culture conditions will it be possible to identify the specific factors responsible for their variable growth characteristics and epiphytic life-style in natural waters.

One final series of laboratory culture studies deserves comment, having been initiated in part because of several observed positive and negative correlations between blooms of different species within the ciguatera dinoflagellate community. For example, Taylor and Gustavson (in press) commented on the inverse relationship between *G. toxicus* and *Ostreopsis* spp. blooms in the Caribbean, while Carlson (1984) found negative correlations between *G. toxicus* and both *P. rhathymum* and *A. carterae* abundance, and positive correlations between *P. rhathymum* and *P. concavum* in a major study in the Virgin Islands. In a subsequent series of laboratory experiments, Carlson (1984) demonstrated that *P. concavum* and *G. toxicus* produce ectocrines which inhibit each other's growth in bialgal culture. [*Gambierdiscus toxicus* actually produced a substance which was auto-inhibitory in batch cultures (Carlson, 1984).] Thus filtrates of *P. concavum* contained substances which were allelopathic to *G. toxicus* and stimulatory to *P. rhathymum,* but with little or no effect on re-inoculated *P. concavum* cells. This is similar to results from studies showing that ectocrines from other dinoflagellates can affect the growth of co-occurring diatoms and cyanobacteria (Pincemin, 1971; Uchida, 1981). Carlson speculated that the functional role of the ciguatera toxins may be to act as ectocrines which would enable a species to compete successfully with other epiphytic dinoflagellates and diatoms for space. Indeed, a tantalizing piece of preliminary data was recently presented which suggests that a maitotoxin fraction from *G. toxicus* prevented benthic diatoms from adhering to glass cover slips (D. G. Baden, reported in Hall and Shimizu, 1985).

OVERVIEW

The general status of ecological research on the ciguatera dinoflagellates is that of a collection of observations and results that suggest certain relationships between the toxic dinoflagellates and their environment, their macroalgal hosts, and each other. These relationships are not documented thoroughly, however, and many contradictions or inconsistencies are apparent. This is true despite a long series of field studies by Yasumoto, Bagnis, and co-workers in the Pacific and extensive studies of the Virgin Islands by Carlson, Tindall, Taylor, and others. The incomplete nature of research into this phenomenon is in no way a reflection of the quality of the research by these workers. Indeed, their perseverance and methodical approach is to be commended. Instead, we must recognize that the causative organisms were first discovered only ten years ago, so field and laboratory methodologies are all relatively new. In addition, these organisms grow slowly in culture, they have unusual and varied requirements for culture medium, and they are both spatially and temporally patchy in the natural environment.

Considerably more effort has been invested in research into the chemical characteristics of the ciguatera toxins, but again the knowledge is incomplete. This is due to a different set of problems, the most important of which is the low concentration of the toxins in fish and in cultured dinoflagellates, making it difficult to obtain sufficient purified material for chemical analysis. Additional problems include the inconsistent and potentially incomplete chemical separation of the toxins from each other by different workers and the lack of a specific assay for each of the toxins.

Despite these limitations, much progress has been made and more is certainly forthcoming as ongoing work builds upon this preliminary base of knowledge. Certain areas of research seem especially important at this juncture. On the chemical side, there is a genuine need for the development of assay methodologies which will distinguish ciguatoxin, maitotoxin and okadaic acid from each other following a simple extraction procedure. Many are hopeful that the desired degree of specificity will come with the immunochemical assays that are now being developed. Concurrent with research into assay methodologies, standard extraction and purification procedures are needed so that the problems with impure preparations can be avoided. Once such procedures are established, it should then be possible to determine whether ciguatoxin is produced by *G. toxicus* (or other species) in laboratory culture and to study how that toxicity varies with growth conditions. If ciguatoxin production in culture can be verified and then maximized, the shortage of purified toxin that has limited progress so severely can rapidly be eliminated.

In addition to culture efforts directed at toxin production by the ciguatera dinoflagellates, considerable laboratory effort is needed to determine their nutritional requirements for, and sensitivities to, certain naturally occurring organic and inorganic compounds. If we knew why some dinoflagellate species choose to live attached to macroalgae, we might then have insights into the factors that regulate population abundance, especially those resulting in distinct seasonal cycles. Likewise, an understanding of the chemistry of the seawater surrounding the cells may lead to an appreciation of the factors that limit these species to areas free from terrestrial runoff. The production of ectocrines and other substances that affect co-occurring species is surely a fertile area for investigation and is one that may well explain certain population fluctuations. The time also seems right for the use of established techniques developed for the study of the effects of fluid flow on small organisms, with the eventual goal of learning the extent to which physics determines dinoflagellate/host preferences. In this case, and in virtually all of the above research directions, the more we move towards well-controlled laboratory cultures, flumes, and mesocosms, the better

will be our quantitative understanding of these enigmatic organisms. Field studies are certainly of great value, but one of the lessons of the last decade has been that the natural habitat of the ciguatera dinoflagellates is complex and highly variable in both space and time and thus gives up its secrets very slowly.

ACKNOWLEDGMENTS

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